

Evaluation of Chlorhexidine Substantivity on Human Dentin: A Chemical Analysis

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Abstract

Introduction: The aim of this study was to investigate the substantivity of chlorhexidine (CHX) solution and gel within a root canal system for 24 hours, 30 days, and 90 days. **Methods:** Forty-five extracted human anterior teeth were used for this study. The samples were divided into 3 groups according to the chemical auxiliary substance used to perform the root canal preparation: group 1, 2% liquid CHX; group 2, 2% gel CHX; and group 3, distilled water (the control group). The working length was determined by inserting a #10 K-file into the canal up to the moment its tip was seen in the apex foramen and then withdrawing it 1 mm. The roots were prepared up to the instrument #45. Longitudinal grooves were carved on the free surfaces of the roots, providing 2 halves of each root and resulting in 30 samples per group. Each group was randomly divided into 3 subgroups ($n = 10$), and substantivity was evaluated after 24 hours, 30 days, and 90 days of incubation. The amount of CHX (in micrometers) was measured through reverse-phase high-performance liquid chromatography. Statistical analysis was performed by analysis of variance and the Tukey test for post hoc comparisons ($\alpha = 0.05$). **Results:** The control group showed no substantivity. Significant amounts of CHX solution and gel remained retained in dentin substrates independent of the time of incubation ($P < .05$). CHX solution showed a higher substantivity than CHX gel, with the exception of groups incubated for 90 days. The decreasing amounts of retained CHX inside the canal were for 24 hours >30 days >90 days for CHX solution and 24 hours >30 days $\geq$ 90 days for CHX gel. **Conclusions:** The results of this study indicate that CHX solution and gel are retained in root canal dentin for up to 90 days. (*J Endod* 2012;38:1249–1252)

Key Words

Chlorhexidine, dentin, substantivity

A major goal of endodontic therapy is to eliminate bacteria from the root canal system to create an environment that is most favorable for healing (1). This is achieved through mechanical cleaning and shaping as well as irrigation with antibacterial agents (2, 3). Sodium hypochlorite (NaOCl) in a concentration range from 0.5% to 5.25% has traditionally been used for irrigation during root canal treatment because of its antimicrobial activity and the ability to dissolve organic matter (2). Its antimicrobial property is proportional to the chemical concentration. In low concentrations, it is ineffective against specific microorganisms (4, 5), and severe irritations have been reported when such concentrated solutions were forced into the periapical tissues (6, 7).

Chlorhexidine digluconate (CHX) has been suggested as an auxiliary irrigant substance in endodontic treatment because of its antimicrobial activity (8, 9) and substantivity (10–13). Studies comparing the antimicrobial effectiveness of NaOCl and CHX have reported that CHX is more effective (11, 14), and others observed no significant difference between them (15–17). Furthermore, CHX does not affect the bond strength of resin composite restorations to the coronal dentin (18) or the root canal sealer to dentin (19). According to Moreira et al (20), CHX is an auxiliary chemical substance that does not interfere with collagen present in the organic matrix of root dentin. In this way, it maintains the quality of the dentin substrate for posterior filling or restoration of the tooth with resin-based materials. Recent studies showed that CHX improves the longevity of composite adhesive bonding to dentin by inhibiting hybrid layer collagen-degrading enzymes called matrix metalloproteinases (MMPs) (21–23), thereby offering a valuable alternative to clinicians who seek to delay the degradation process of adhesive restorations.

Unlike NaOCl, CHX is capable of remaining in the dentin. This remaining material imparts long-lasting effects on dentin, which is termed substantivity (10–13). Dametto et al (11) showed that 2% CHX (gel and liquid) kept low colony-forming units of *Enterococcus faecalis* for 7 days after the biomechanical preparation. In an *in vivo* study, Leonardo et al (10) evaluated the antimicrobial substantivity of 2% CHX in teeth with pulp necrosis and radiographically visible chronic periapical lesions. They showed that CHX prevented microbial activity with residual effects in the root canal system for up to 48 hours after application.

Thus, the aim of this study was to investigate the substantivity of CHX gel and solution within a root canal system for 24 hours, 30 days, and 90 days by chemical analysis. The tested null hypotheses were that (1) CHX gel and solution have substantivity and (2) the substantivity is time dependent.

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Materials and Methods

Specimen Preparation

This study was submitted to the Science and Ethics Commission. Teeth were stored in 0.02% thymol solution, prepared within 1 month of extraction, and autoclaved before use. Forty-five freshly human extracted anterior teeth with similar root segments and fully developed apices were selected. The root surfaces were examined for the absence of fracture lines or anatomic irregularities and were discarded if any of these features were found. All teeth were extracted from patients between the ages of 20 and 35 years. None had initial endodontic treatment, and the apical foramen was sealed with composite resin (B0.5, Z250; 3M ESPE, St Paul, MN). Each tooth was decoronated below the cementoenamel junction perpendicular to the longitudinal axis using a slow-speed, water-cooled diamond disc (Isomet 2000; Buehler Ltd, Lake Bluff, IL). The roots were cut to a uniform length of 15 mm from the apical end. After the procedures, the root canals were irrigated with distilled water, and the pulp tissue was removed with a #15 K-file (Maillefer, Ballaigues, Switzerland). The working length was determined by inserting a #10 K-file (Maillefer) into the canal up to the moment its tip was seen in the apex foramen and then withdrawing it 1 mm.

Chemomechanical Preparation

All teeth were instrumented with the crown-down technique using rotary nickel-titanium K3 instruments (SybronEndo, Glendora, CA) at a constant speed of 350 rpm up to a #45.02 file to the working length. The apical stop was established using files up to size 45 followed by a step-back instrumentation, which ended after the use of 3 files larger than the last file used for the apical preparation. Stepping back ended after the use of 3 files larger (K-file 60) than the file that prepared the apical stop. This technique was described previously by Berber et al (24).

The following regimen was used. In group 1, before a new instrument, the canal was filled with 2% CHX solution (Natufarma, Passo Fundo, RS, Brazil). The root canal was filled with CHX using a 3-mL syringe with a 19-G needle. The needles were centered within the canal 3 mm short of the working length. Each instrument was used for 3 minutes in the root canal. After the use of each instrument, 5.0 mL distilled water was used as an irrigating solution with a 5-mL syringe and a 30-G needle 3 mm short of the working length. In group 2, the same protocol was used as in group 1; however, 2% CHX gel (Natufarma) was used as an auxiliary chemical substance. In group 3 (the control group), the same protocol was used as in group 1; however, distilled water was used as an auxiliary chemical substance. Final irrigation with 2 mL 17% EDTA for 3 minutes followed by irrigation with 5 mL distilled water was performed in order to remove the smear layer (25). After that, all canals were dried with sterile paper points to conclude the protocol.

CHX in groups 1 and 2 was the chemical auxiliary used with the endodontic instrument for root canal preparation. Distilled water was the irrigating solution used to remove CHX and the material originated from the instrumentation of the root canal.

Longitudinal grooves were carved on the free surfaces of the roots with a diamond disk, taking care not to invade the inner part of the root canal. The complete fracture was made with a chisel and hammer, providing 2 halves of each root and resulting in 30 samples per group. The samples were stored at 37°C under 100% relative humidity. Each group was randomly divided into 3 subgroups ($n = 10$), and substantivity was evaluated after 24 hours, 30 days, and 90 days of incubation.

Quantification of CHX

The method for CHX determination was adapted from Rasimick et al (13). The samples were placed in 1- and 5-mL tubes, and extrac-

tion solution (acetonitrile:formic acid 1%, 20:80) was added. The tubes were heated in a water bath at 80°C for 20 minutes and sonicated for 10 minutes. Subsequently, the liquid contents were transferred to 1.5-mL tubes and centrifuged at 6,000 rpm for 15 minutes. The supernatants were diluted 10 times, and 20 μ L was injected into the high-performance liquid chromatography system. CHX was assayed by using an isocratic separation with methanol:water (63:37, v/v). Triethylamine (0.4%) was added to the mobile phase, and the pH was adjusted to 3.7 with chloridric acid. A 1.0-mL/min flow rate was maintained with the diode array detector set at 260 nm, producing a total run time of 8 minutes. Twenty microliters of samples was injected in a high-performance liquid chromatography system equipped with an isocratic pump, diode array detector, degasser, and manual injection system (all high-performance liquid chromatography components and software ChemStation were from Agilent Technologies Inc, Santa Clara, CA). Chromatographic separations were performed using a reverse-phase column (250 $\times$ 4 mm, 5-mm LiChrospher 100 RP-18). The column was protected by a guard column (4 $\times$ 4 mm, 5-mm LiChrospher 100 RP-18; Merck Millipore, Frankfurt, Germany) and was maintained at a temperature of 22° $\pm$ 2°C.

The means and standard deviations of substantivity of CHX solution and gel were calculated in micrometers, and the data were analyzed using 2-way analysis of variance and the Tukey test for post hoc comparisons ($\alpha = 0.05$).

Results

The means and standard deviations are presented in Table 1. The control group showed no substantivity. Significant amounts of CHX solution and gel remained retained in dentin substrates independent of the time of incubation ($P < .05$). CHX solution showed a higher substantivity than CHX gel, with the exception of groups incubated for 90 days. The decreasing amounts of retained CHX inside the canal for CHX solution were 24 hours >30 days >90 days and for CHX gel they were 24 hours >30 days $\geq$ 90 days.

Discussion

Although chemomechanical preparation reduces the bacterial load, complete disinfection is almost impossible to achieve as a result of the complex anatomy of the root canal system (26, 27). In this regard, Peters et al (28) reported that mechanical instrumentation alone left more than 35% of the root canal surface untouched. NaOCl, because of its powerful germicidal and bactericidal properties, is still the most frequently used root canal irrigant (2). However, NaOCl acts only during the instrumentation procedures, but it does not exert any residual antimicrobial activity (11) so the recolonization of persistent microorganisms is not prevented. Upon the initial exposure to CHX, Dametto et al (11) showed that the antimicrobial activity of CHX is at least as effective as NaOCl. In addition, as revealed in this study, its substantive antimicrobial activity offers potential protection of the canal tissues for as many as 7 days after instrumentation. Although NaOCl may be equally effective on the initial exposure, it is not a substantive antimicrobial agent. Because antimicrobial effectiveness is surely the most

TABLE 1. The Amount of CHX (in μ M) Remaining (ie, substantivity) over Time as a Function of the Solution and Gel

	24 hours	30 days	90 days
CHX solution	48.97 $\pm$ 7.85 ^a	13.95 $\pm$ 4.42 ^c	3.38 $\pm$ 1.59 ^d
CHX gel	22.22 $\pm$ 5.19 ^b	5.40 $\pm$ 1.03 ^d	2.03 $\pm$ 1.14 ^d
Distilled water (control)	0.00 $\pm$ 0.00 ^e	0.00 $\pm$ 0.00 ^e	0.00 $\pm$ 0.00 ^e

Different letters indicate a statistically significant difference at the 5% level.

important property required for an irrigant solution to be used during the treatment of teeth with apical periodontitis (17), some investigators have suggested the use of CHX as an auxiliary antimicrobial agent during the biomechanical procedures (9, 11, 14).

CHX is characterized as being a strong base with cationic properties (29). Its efficacy is caused by the interaction of the positive charge of the molecule and the negatively charged phosphate groups on microbial cell walls, thereby altering the cells' osmotic equilibrium. This increases the permeability of the cell wall, which allows CHX molecules to penetrate into the bacteria (30). At a low concentration (0.2%), low-molecular-weight substances, specifically potassium and phosphorous, will leak out of the cell. On the other hand, at a higher concentration (2%), CHX is bactericidal as precipitation of the cytoplasmic contents occurs, which results in cell death (30). Furthermore, CHX adsorbs to surfaces covered with acidic proteins, such as hydroxyapatite, and is gradually released in the form of an active cation (substantivity), justifying its clinical use (10–13). This substantivity was confirmed in this study.

The results of this study indicate that CHX (solution or gel) is retained in root canal dentin for at least 90 days. Therefore, the first hypothesis tested in this study was confirmed. Previous studies that have investigated the substantive properties of CHX have tested for its presence for up to 48 hours (10), 7 days (11), and 8 weeks (12). In addition, according to Rasimick et al (13), groups monitoring decomposition of CHX in water had half-lives of 40 weeks. The half-life of the antimicrobials on dentin is suspected to be largely caused by diffusion of the antimicrobials. These previous studies only analyzed the substantivity of CHX solution. In the current study, the substantivity of CHX solution or gel was measured through reverse-phase high-performance liquid chromatography used to estimate the amount of CHX that is retained in the dentin of the root canal wall. We observed that CHX solution has greater substantivity than CHX gel. This is possibly because of the higher capacity of the solution to penetrate the dentinal tubules.

Moreover, in our study, we observed that CHX substantivity is time dependent. Thus, over time, the amount that remains in CHX on dentin reduces. These results are consistent with other studies (12, 13) and confirm the second hypothesis in the study. There are several factors that might limit the substantivity of CHX. In addition to dentin, other molecules present in the root canal can alter the efficacy of irrigants. Proteins such as serum albumin and collagen as well as killed microbes tend to reduce efficacy. Bacteria undergoing rapid growth tend to be sensitive to irrigants, whereas stressed microbes are usually resistant. These mitigating factors might shorten the lifespan of CHX (13).

Chemical substances used during biomechanical preparation of root canals may alter the structure of dentin, mainly collagen. This may interfere with the penetration of monomers to within the demineralized dentin structure, consequently putting the quality and durability of direct restorations and fiber post-cementation at risk (31, 32). Details of this information are important because of the necessity of sealing endodontically treated teeth using resin-based materials or when using a resin sealer for root canal obturation. Moreira et al (20) showed that when bovine root dentin was exposed to 5.25% NaOCl for 30 minutes, whether it was associated or not with 17% EDTA, a morphologic disorganization and structure loss of the dentin organic matrix were observed closer to the root canal. NaOCl causes dentin degeneration because of the dissolution of collagen by breaking down bonds between carbon atoms and disorganizing the protein primary structure (32). On the other hand, 2% CHX, whether associated or not with 17% EDTA, did not promote morphologic structure alterations of the dentin organic matrix. Hence, these results indicate that 2% CHX is an auxiliary chemical substance that does not interfere with the collagen present in the organic matrix of root dentin; thus, it

maintains the quality of the dentin substrate for posterior obturation or restoration of the tooth with resin-based materials.

Furthermore, CHX also has potent anti-MMP-2, -8 and -9 activity, resulting in beneficial effects on the preservation of resin-dentin bonds (21). MMP-2, -8, and -9 have been detected in human crown dentin (33, 34) and radicular dentin (35), and their release and activation contribute to the organic matrix degradation along resin-dentin-bonded interfaces (36, 37), compromising the durability of adhesive restorations over time. Cecchin et al (22, 23) showed that the pretreatment of root dentin with CHX kept the adhesive longevity for 12 months because the bond strength of the anatomic post to the root dentin remained high and unchanged in relation to the immediate control groups. Carrilho et al (36) and Ricci et al (37) showed *in vivo* that the protection of CHX application against the degradation of the coronal adhesive interface lasted for up to 14 and 12 months, respectively, after the establishment of resin-dentin bonds. According to Carrilho et al (21), the long-term action of CHX can be explained by its confinement to the adhesive interface because it is possible that the removal by the dentinal fluid outflow is minimized by the formation of resin tags that obliterate the tubules. In addition, the adhesive monomers that envelop the collagen fibrils treated with CHX as well as the presence of an adhesive layer on the hybrid layer can contribute to the preservation of CHX at the interface and prolong its inhibitory action.

CHX has been suggested as an endodontic intracanal irrigant by a number of authors because of its cleansing ability (8–11), antimicrobial activity (11, 14–17), and substantivity (10–13). Furthermore, Tanomaru Filho (38) evaluated the apical and periapical repair after endodontic treatment of teeth with pulp necrosis and a chronic periapical lesion in dogs using 5.25% NaOCl or 2% CHX as the irrigating solution. These authors observed that the irrigation with CHX solution resulted in better repair than NaOCl. Moreover, the recent research indicates that the substantivity of CHX to dentin may play a paramount role in the inhibition of collagen-bound proteases and, consequently, in the stability of CHX-treated resin-bonded interfaces (22, 23, 36, 37). Although these substantive and antimicrobial properties of CHX found here are promising, it does not have the tissue-dissolving properties of NaOCl (39). Furthermore, the association between substances should be better investigated. Although the association between NaOCl and CHX is not indicated by the possibility of the formation of a precipitate and color change of dental structure (40), Baca et al (41) suggested an association between CHX and cetrimide. These authors showed that the combination of CHX and cetrimide would be an effective alternative final irrigation regimen given its antimicrobial action over time. Therefore, the impact of the use of CHX as an endodontic irrigant associated with mechanical instrumentation should be evaluated by clinical trials.

Conclusion

The results of this study indicate that CHX solution and gel are retained in root canal dentin for up to 90 days.

Acknowledgments

The authors deny any conflicts of interest related to this study.

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